

GENETIC MARKERS TO DISTINGUISH
AMONG WEST COAST LAMPREY
SPECIES
AND THE POPULATION STRUCTURE
OF THESE SPECIES

Margaret F. Docker, University of Windsor

Collaborators: S.B. Reid, D.F. Markle,
C.M. Lorion, D. Goodman, G.R. Haas

OUTLINE

- Use of genetic markers in lampreys
- Basic molecular genetic tools
 - PCR
 - Mitochondrial RFLP assays
- Species-specific markers
 - e.g., Pacific lamprey vs. western brook lamprey
- Intraspecific genetic variation
- Future work

USE OF GENETIC MARKERS

1. Species-specific markers necessary for species ID of most ammocoetes
 - To determine relative abundance and distribution
 - Easier to survey ammocoetes than juveniles and adults
 - e.g., Pacific lamprey vs. western brook and river lamprey in coastal streams

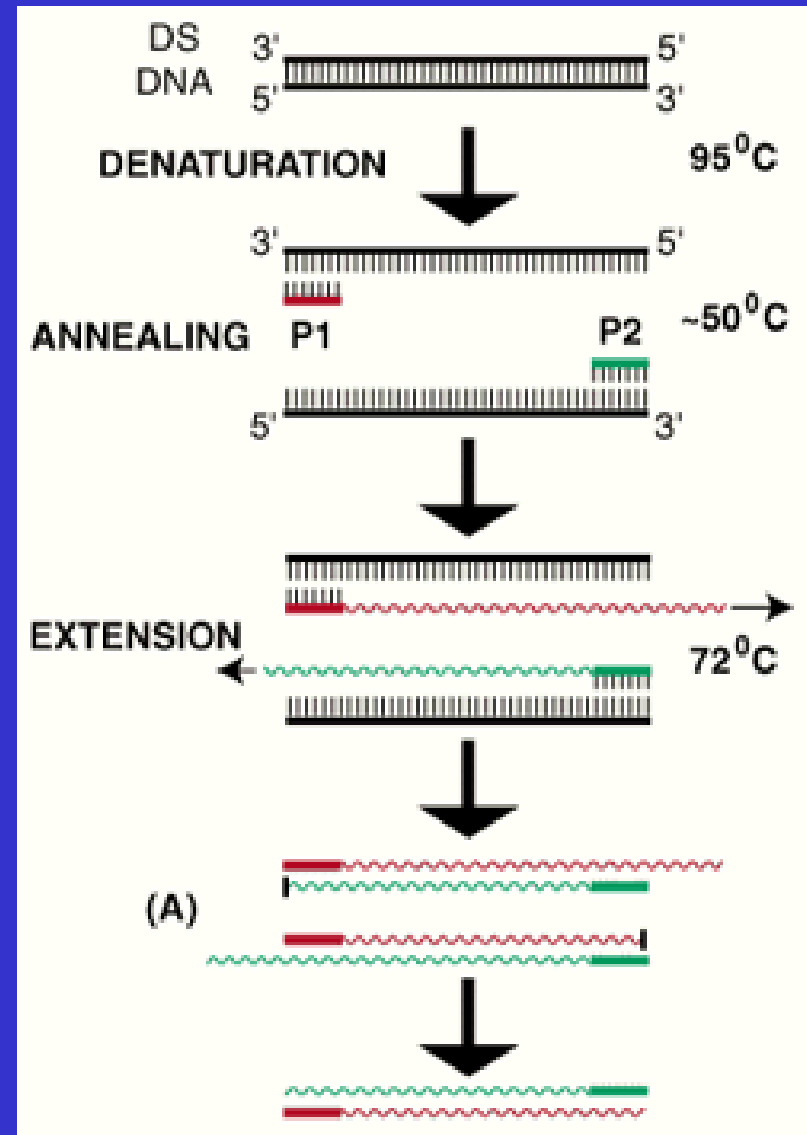
2. Intraspecific markers useful to study genetic structure of populations

- Do populations from different geographic locations differ genetically?
- Does population structure differ among species?
- Do different co-occurring types differ genetically?
- And at what point might “intraspecific” variation be great enough for types to be considered separate species?

MOLECULAR GENETIC “TOOLBOX”

PCR, Polymerase Chain Reaction

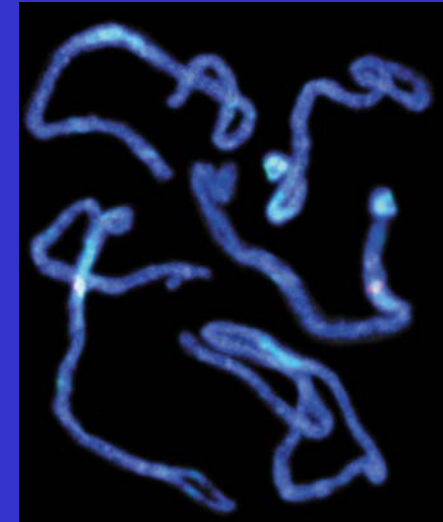
- Foundation of all other techniques
- Amplifies any stretch of DNA that is flanked by synthetic oligonucleotide primers (P1 and P2)



- PCR is highly sensitive
 - Amplifies gene of interest millionfold
 - Requires very small amount of tissue
 - Useful in forensics, conservation biology
- Only limitation is that DNA sequence of flanking region must be known to design primers
- Hence usefulness of mitochondrial DNA

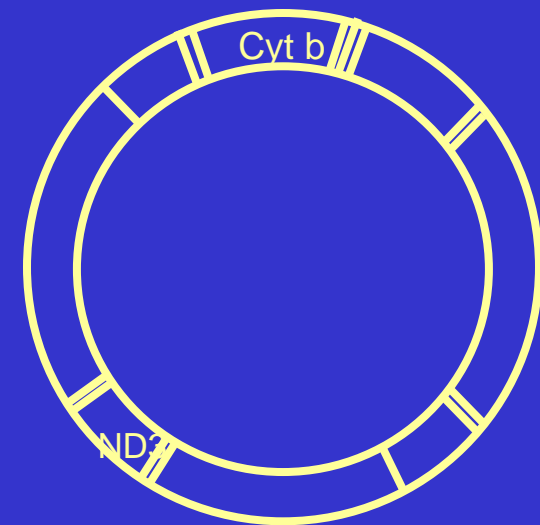
Nuclear Genome:

- Large and complex (3.2 billion bp in humans)
- Primers often species-specific
- And lamprey genome virtually unknown



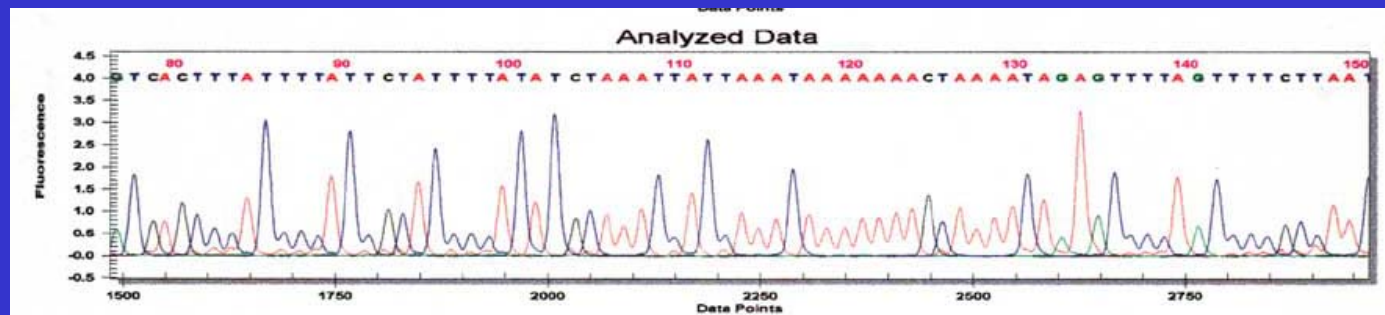
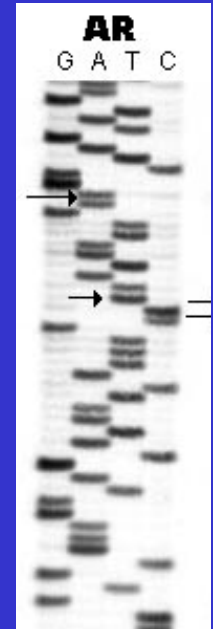
Mitochondrial DNA:

- Small genome (17,000 bp)
- Conserved gene content
- Can design “universal” primers from other vertebrate sequences
- But it is maternally-inherited



DNA Sequencing

- Can determine DNA sequence of PCR fragments
- Used to be very time-consuming and technically involved
- But automated sequencing now much faster and less technically demanding



- Still expensive but can be used as starting point for faster screening methods for high throughput

RFLP, Restriction Fragment Length Polymorphism

- Can quickly screen PCR products for specific sequence differences
- Using restriction enzymes that will cut DNA only at specific recognition sites

e.g.,	<i>AluI</i>	AGCT
	<i>BamHI</i>	GGATCC
	<i>EcoRI</i>	GAATTC
	<i>HaeIII</i>	GGCC
	<i>RsaI</i>	GTAC

Markers for Species ID

1. Pacific lamprey (*Lampetra tridentata*) and western brook lamprey (*L. richardsoni*)
 - Several PCR-RFLP assays quickly distinguish between these species
 - Based on differences in their cut patterns=*restriction fragment length polymorphism*
 - e.g., Amplify cyt *b* fragment
 - A. Digest with *Bsa*AI restriction enzyme
- Will cut only TACGTA

Western brook lamprey

Pacific lamprey

....TAC GTA....

....TACGAA....

- Size differences in PCR products are visible on agarose gel

*Bsa*AI

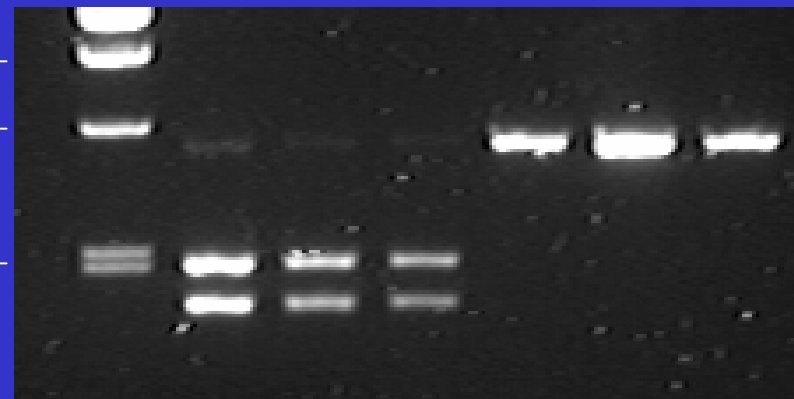
750 bp —

500 bp —

250 bp —

*ayresi /
richardsoni*

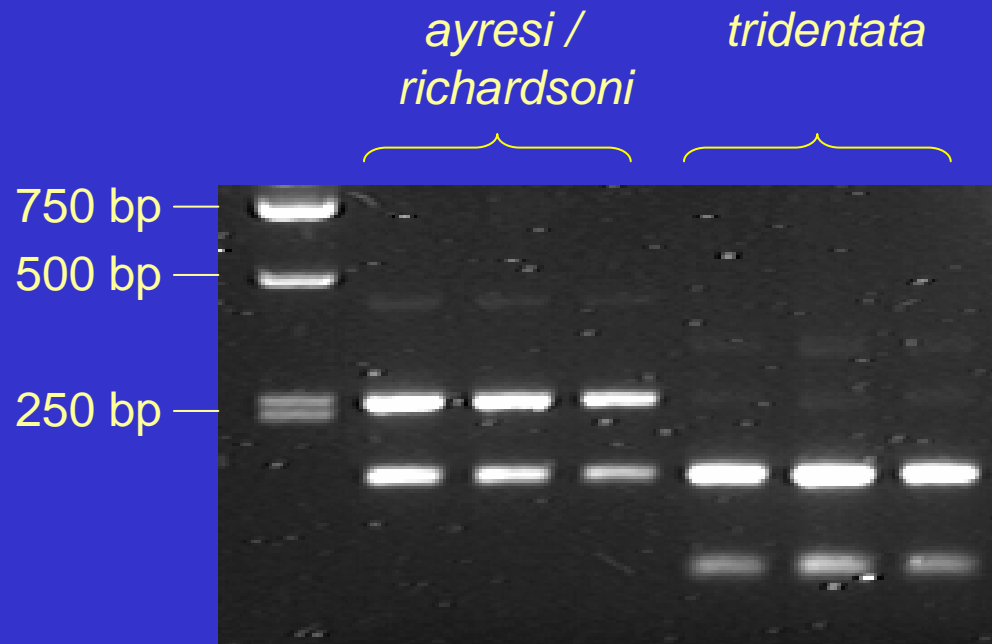
tridentata



B. *Dde*I (CTNAG)



C. *Hae*III (GGCC)

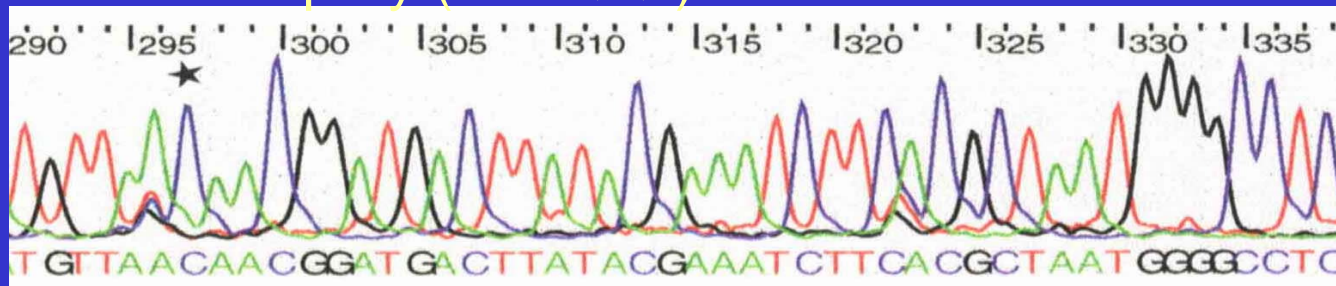


- But cannot distinguish between western brook lamprey and river lamprey (*L. ayresii*)
- Paired species genetically indistinguishable
- River lamprey thought to have limited distribution
- So assay generally useful for distinguishing Pacific and western brook lamprey
- However, an assay that could recognize river lamprey ammocoetes might demonstrate that species more abundant than suggested by adult records alone

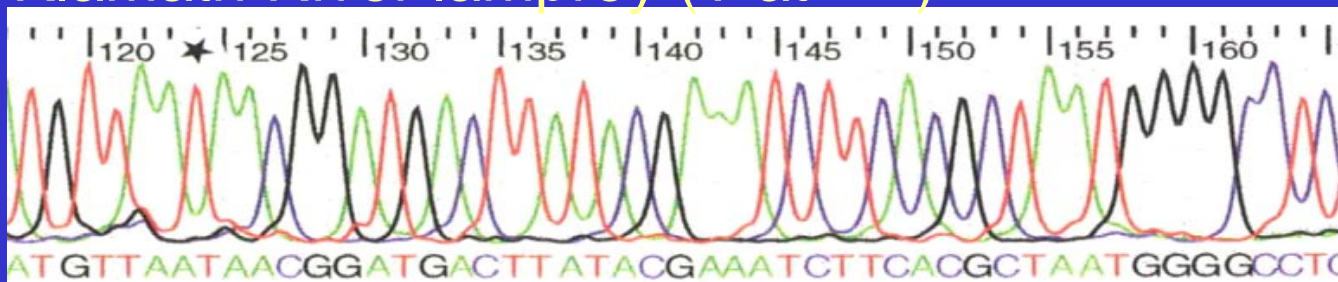
2. Anadromous Pacific lamprey (*Lampetra tridentata*) and Klamath River lamprey (*L. similis*)

- Both occur in lower Klamath R
- Morphologically indistinguishable as larvae
- But genetically distinct (at 1/384 bp of cytochrome *b*)

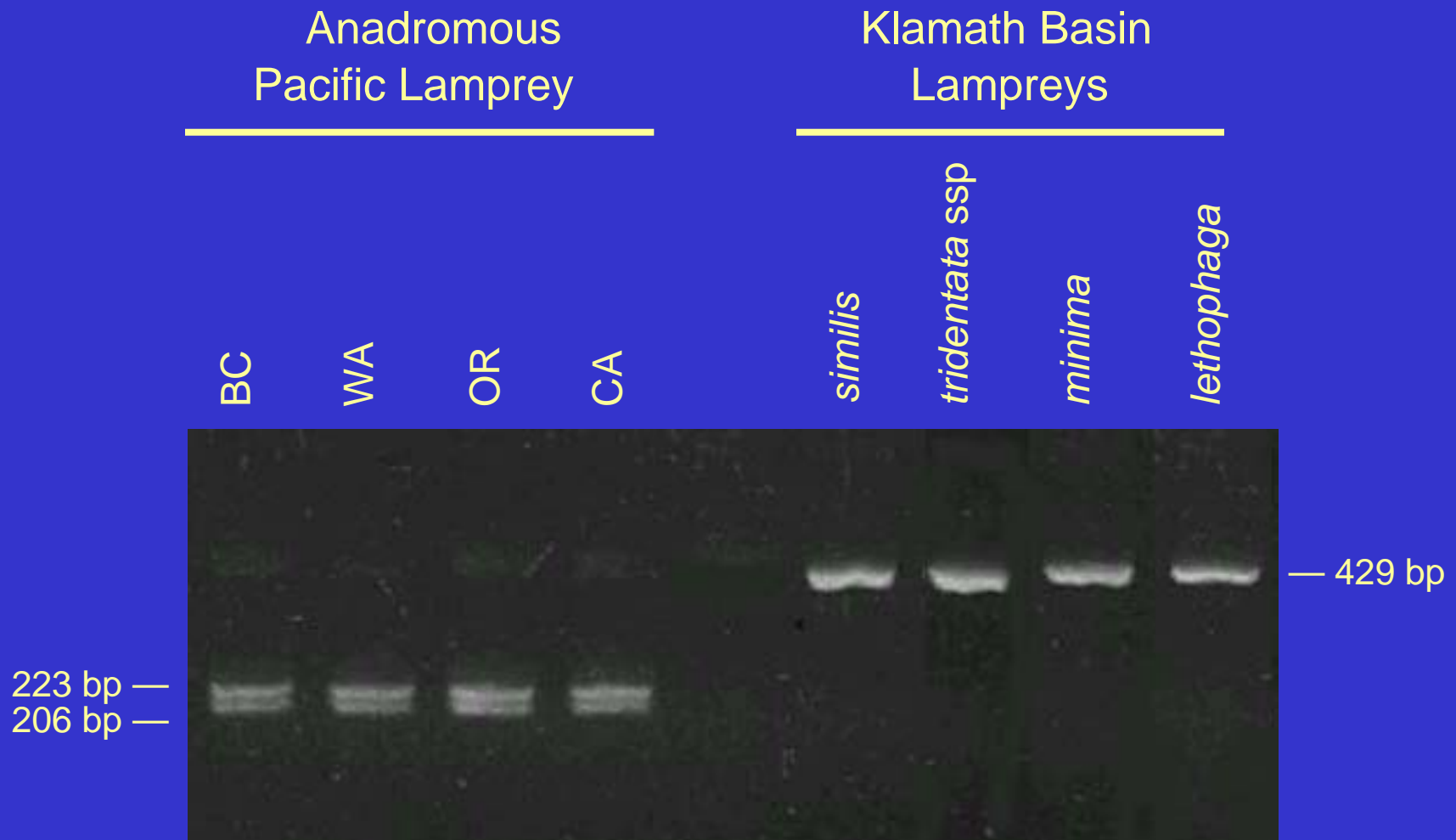
Pacific lamprey (C at ★)



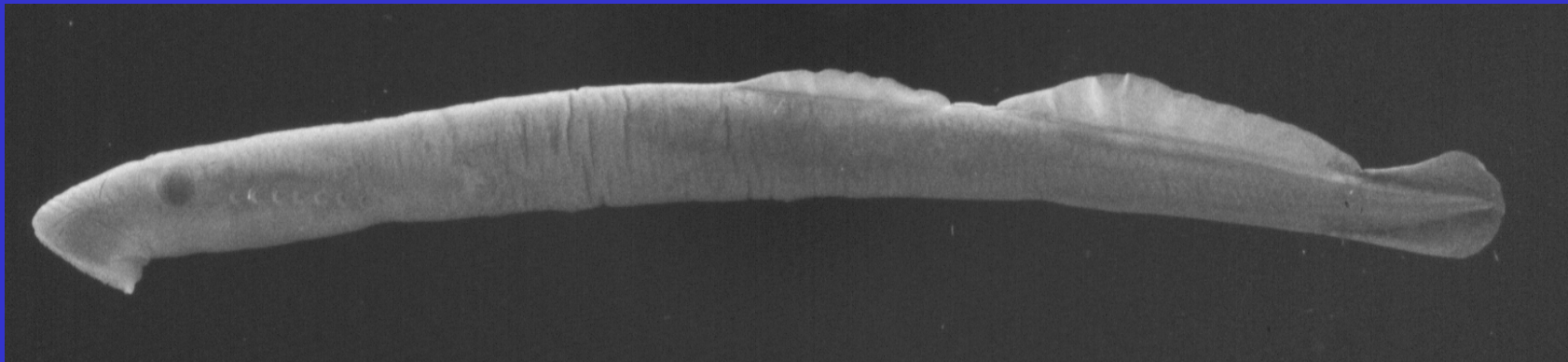
Klamath River lamprey (T at ★)



- Digest cyt *b* fragment with *HpaI* restriction enzyme
- Will cut only GTTAAC



- RFLP distinguishes anadromous Pacific lamprey from ALL Klamath Basin lampreys
- Including dwarf 'landlocked' Pacific lamprey in Upper Klamath Lake
- Considered subspecies of *L. tridentata*
- But genetically very distinct



Lampetra tridentata ssp.

- However, this assay cannot distinguish among the Klamath Basin species, including:
 - *L. minima* (Miller Lake lamprey)
 - *L. lethophaga* (Pit-Klamath brook lamprey)
- But nonetheless useful since Klamath River lamprey only one that occurs with anadromous Pacific lamprey
- Can other assays differentiate the different Klamath Basin species?
- Sequenced 384 bp cyt *b* in >200 lampreys

Coastal BC, WA, OR, CA:

A Anadromous Pacific Lamprey

Lower Klamath River:

A Anadromous Pacific Lamprey

B Klamath River Lamprey

Upper Klamath Basin (6 Sub-Basins):

A_{L1} Landlocked Pacific Lamprey (UKL)

B Klamath River Lamprey

C Miller Lake Lamprey

D Pit-Klamath Brook Lamprey

Pit River System:

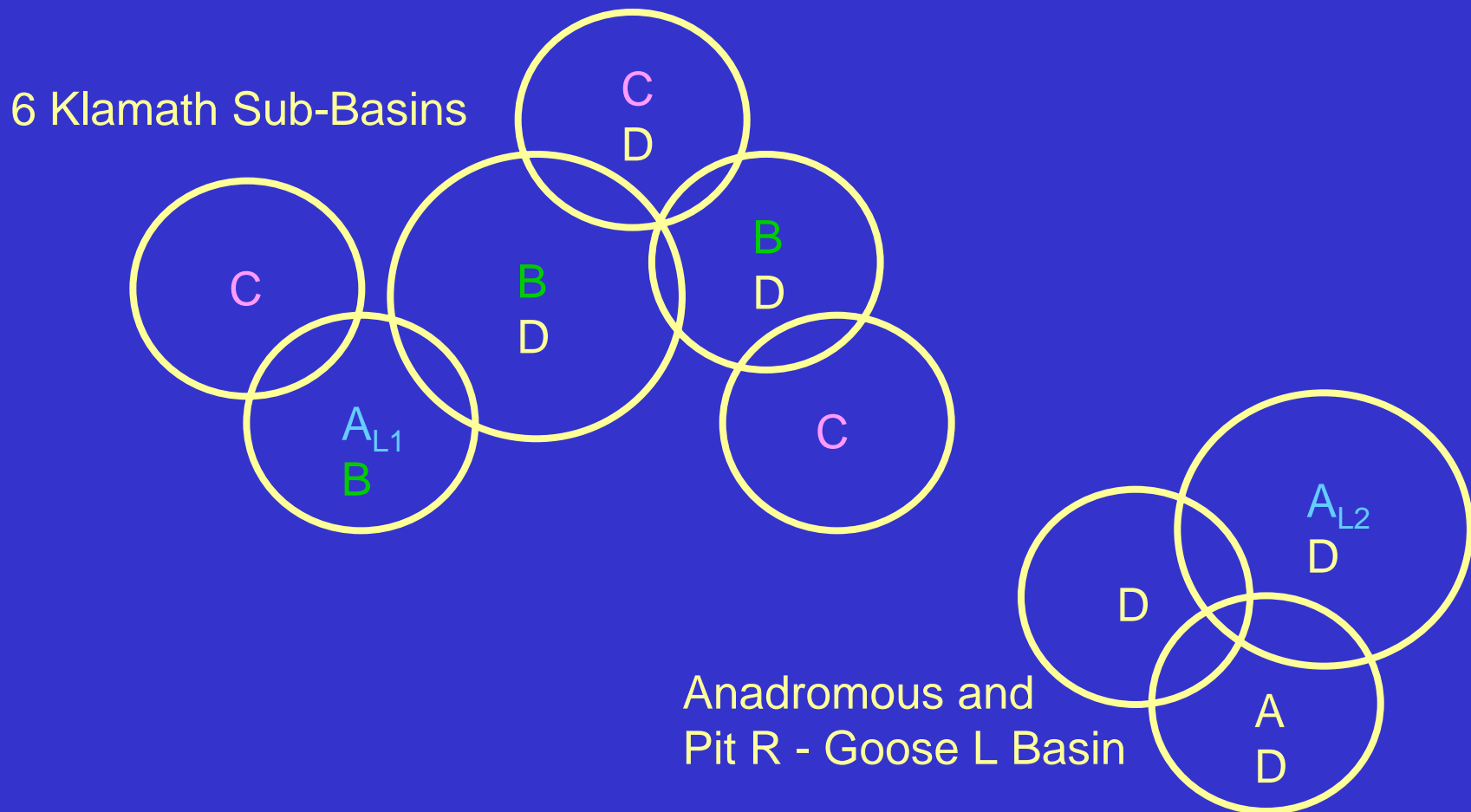
D Pit-Klamath Brook Lamprey

Goose Lake Basin:

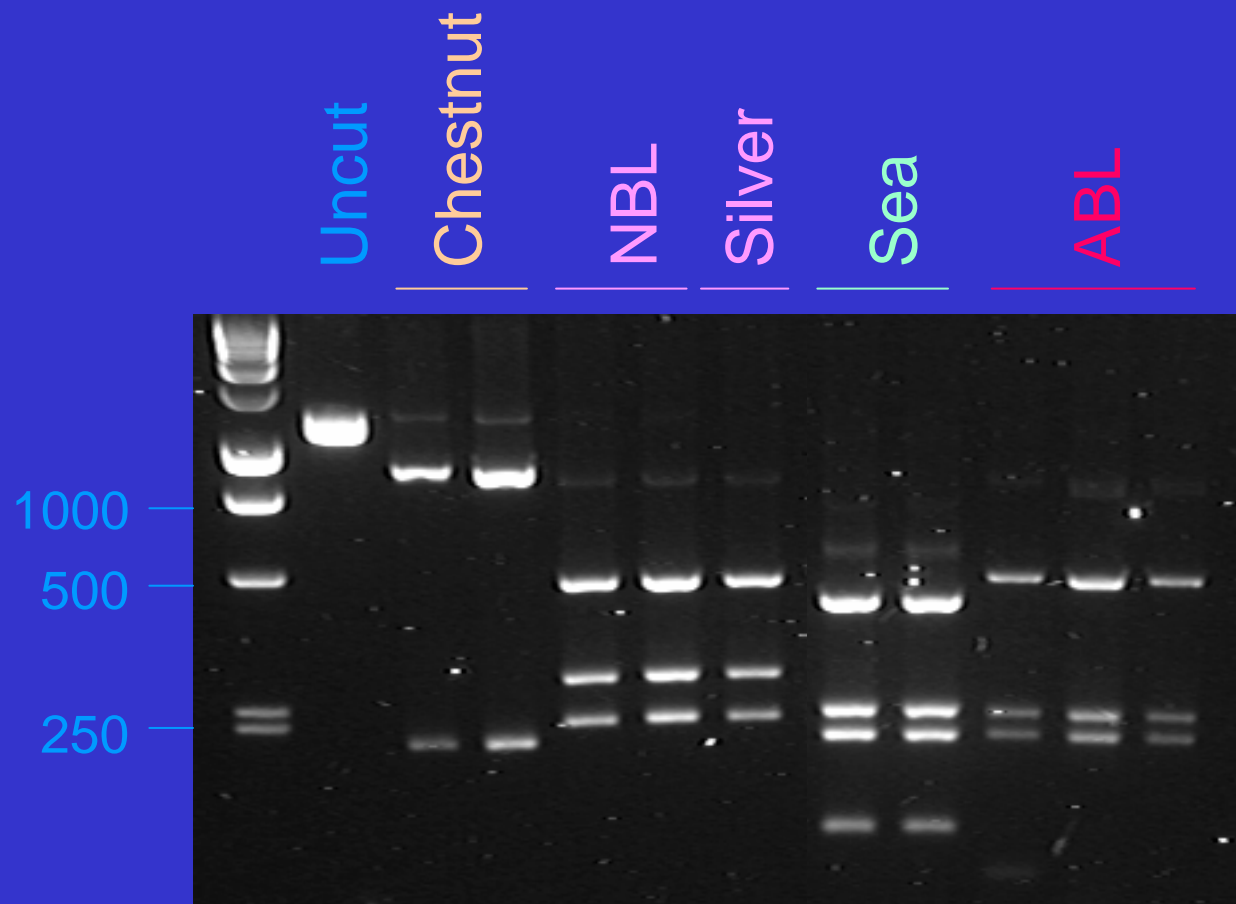
A_{L2} Landlocked Pacific Lamprey (Goose Lake)

D Pit-Klamath Brook Lamprey

- NO diagnostic differences between species
- Genetic groupings are according to geographic location rather than species



- Similarly, RFLP distinguishes among 4 of 5 Great Lakes lamprey species
- But cannot differentiate between northern brook and silver lampreys

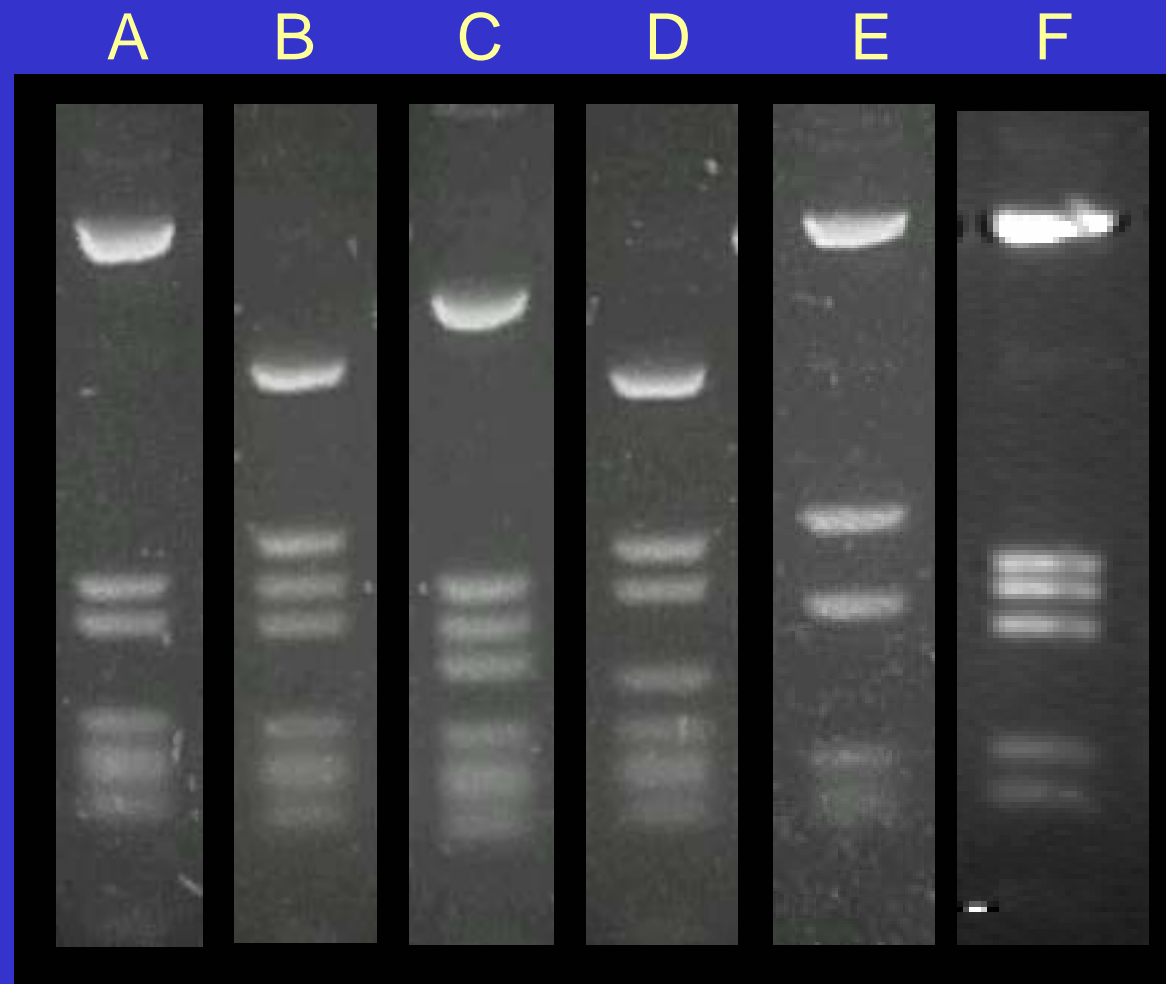


- Perhaps subtle differences between 'paired' and 'satellite' species not evident in only 384 bp
- Sequenced >3,500 bp in west coast species, >11,000 bp in Great Lakes species
- Two important findings:
 - Still no interspecific variation within basins or sub-basins!
 - But lots of intraspecific variation

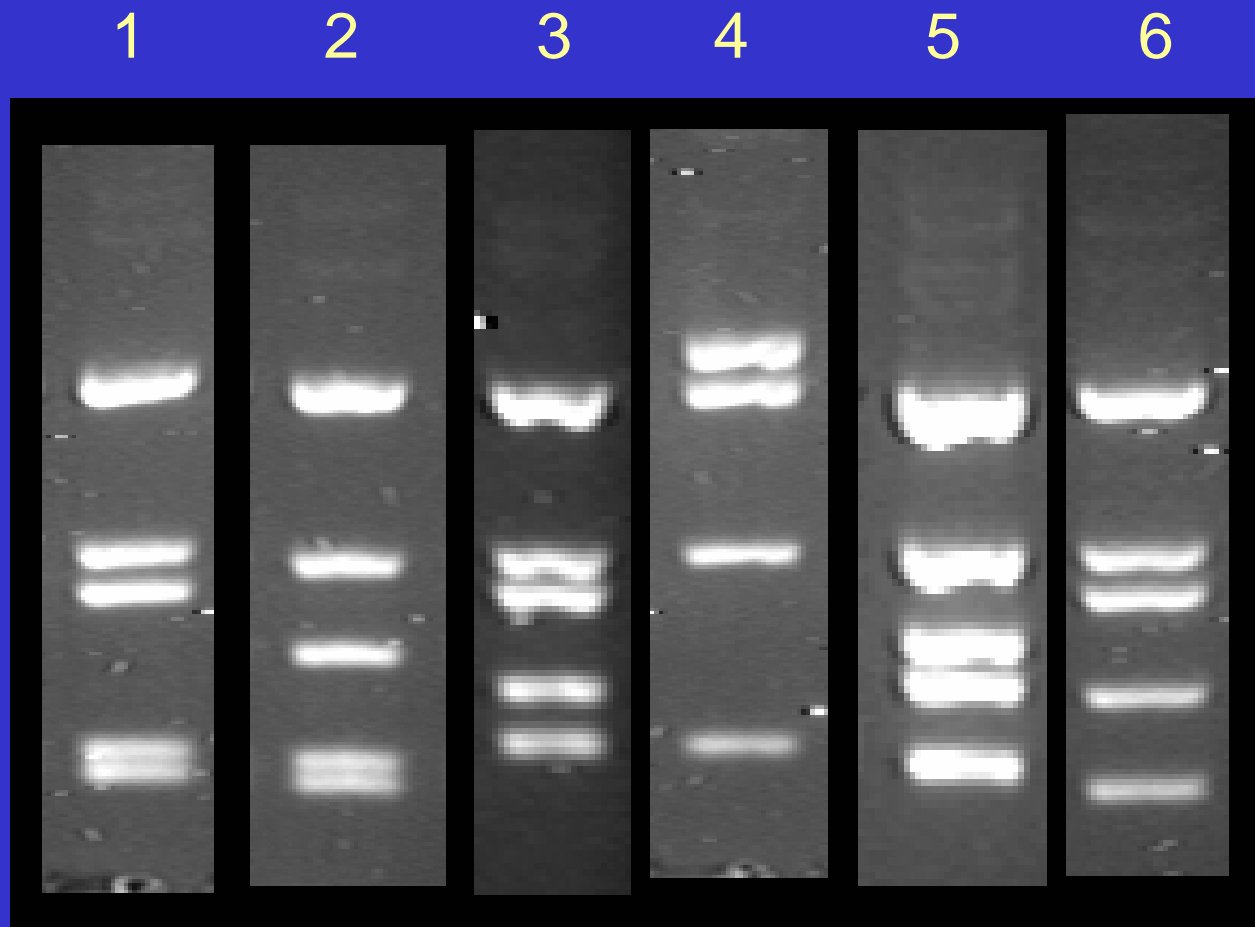
Markers to Study Population Structure

- Sequenced >3,500 bp in *cyt b*, ND1, ND2, ND3, ND5 genes in:
 - Anadromous Pacific lamprey
 - Klamath Basin lampreys
 - Pit R and Goose L lampreys
- Dozens of variable sites
- 13 RFLP assays to survey 21 of these sites
- Permitting high throughput (550 lampreys)
- To study genetic differences among populations

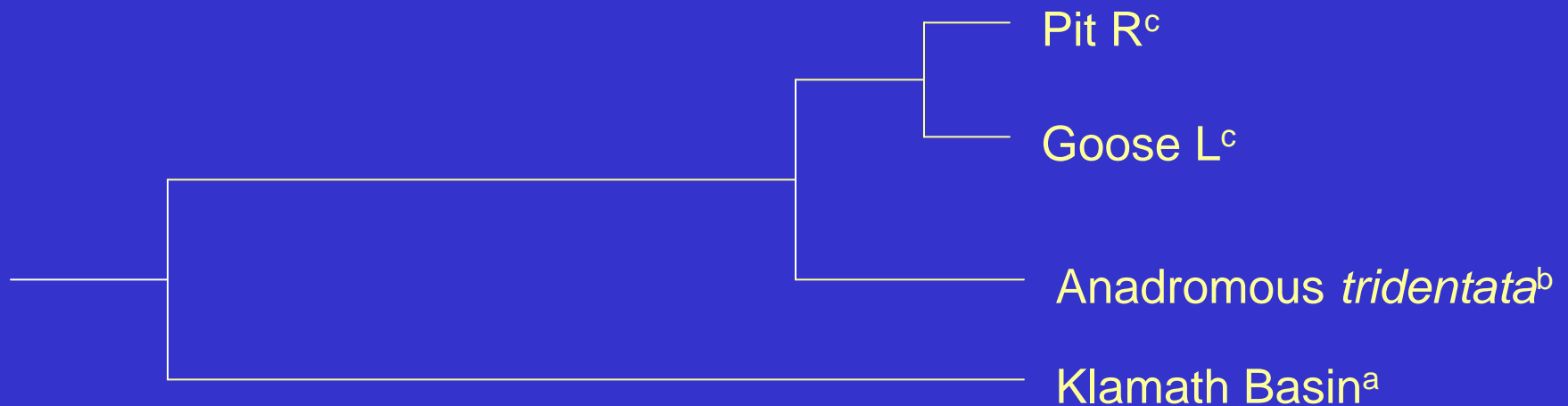
- e.g., ND1/ND2 *Hinf*I RFLP
- 6 different cut patterns representing 5 variable sites



- e.g., ND1/ND2 *Hae*III RFLP
- 6 different cut patterns representing 4 variable sites

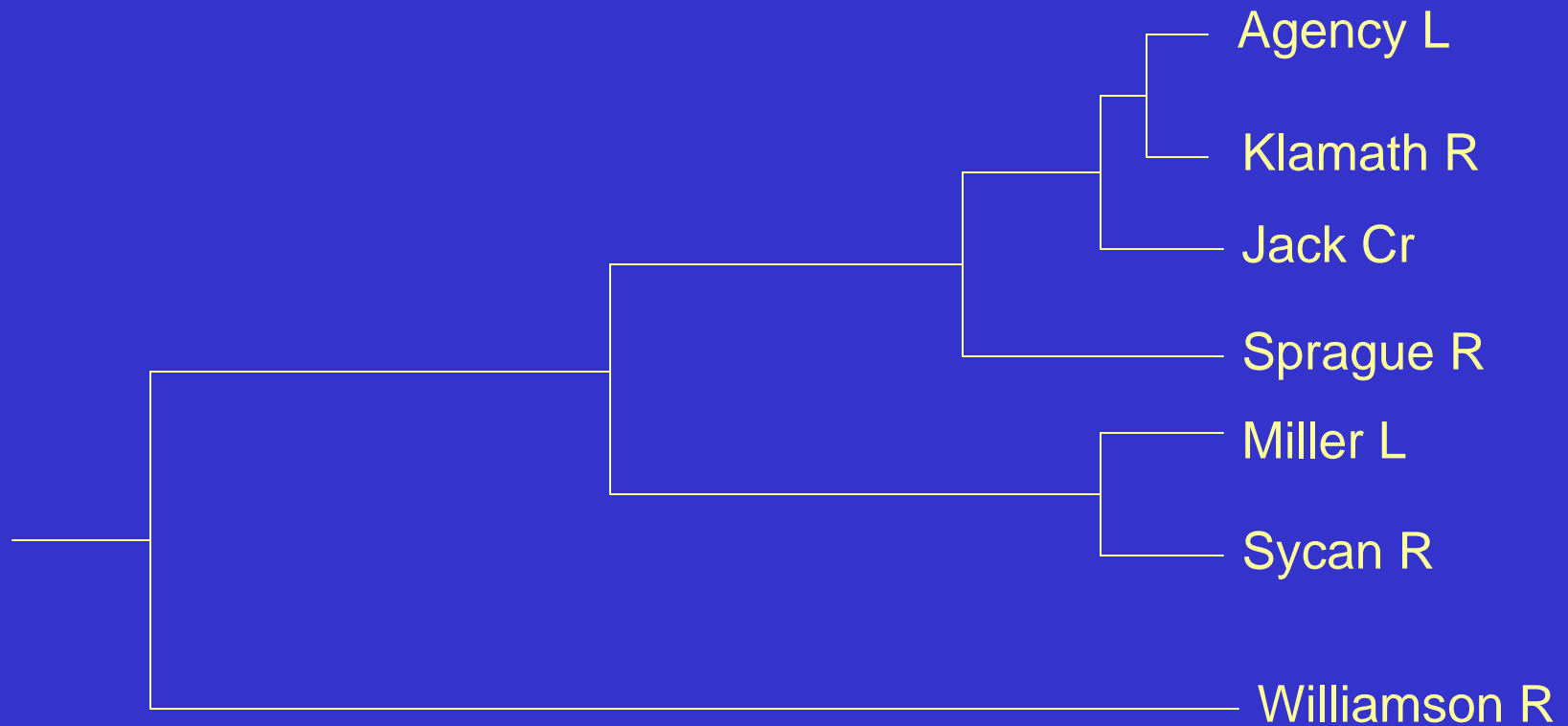


- Combined RFLP results produce 25 different genetic types ('haplotypes')
- Which again show the Klamath Basin lampreys to be distinct (no shared haplotypes)
- Frequency differences between anadromous Pacific lamprey and Pit R, Goose L lampreys



Nei's (1972) original genetic distance, UPGMA

- Can also study genetic relationships among Klamath sub-basins



Nei's (1972) original genetic distance, UPGMA

- And to study population structure in anadromous Pacific lamprey
- 5 RFLP markers specific to anadromous *L. tridentata*
- Producing 8 haplotypes to date
- Preliminary results show some differences between northern and southern populations
- Rare haplotypes in OR, CA absent in BC, WA
- Being used by Damon Goodman, M.S. thesis at Humboldt State University: “A Biogeographical Analysis of *Lamprica tridentata*”

Ongoing and Future Studies

- Is the lack of interspecific variation between 'paired' (e.g., western brook and river lampreys) and satellite species (e.g., Klamath Basin lampreys) because:
 - They aren't species (but rather different morphotypes within a single gene pool)?
 - Or they are very recently evolved species and mtDNA does not provide sufficient resolution?
- Quantify gene flow between these species using high resolution genetic markers (e.g., microsatellites)
- To determine if they reproductively isolated

Microsatellite Markers

- Stretches of 2, 3, or 4 bp repeats
- Number of repeats generally variable
- Highly polymorphic (e.g., 10-50 alleles per population)

Primer

ATAGTTAGCACGACGTAAAGAATTGACCA TAGCTGCAAGTCC
GTAGGACGATTCGCACGGTGAAGATA TCTGTCCAGCTGT CG
CTAGATTGGGGGGGGGG G A G A G A G A G A G A G A G
CTC GTG CT GG CTA CG AGG AG TT C GC GTTGCGTGTCTACACG

Primer

- Can infer number of repeats from size of PCR product
- Using PCR primers that flank repeat region

But:

- Microsatellite markers hard to develop
- Generally applicable only to that species or genus
- Not universal primers like mtDNA
- Developed and testing 11 microsatellite primers in Pacific lamprey
- And David Close (MSU) using some developed for sea lamprey
- Test in other west coast species

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